

# O2k-Protocols SOP: O2k Quality Control 1

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## O2k Quality Control 1: Polarographic oxygen sensors and accuracy of calibration

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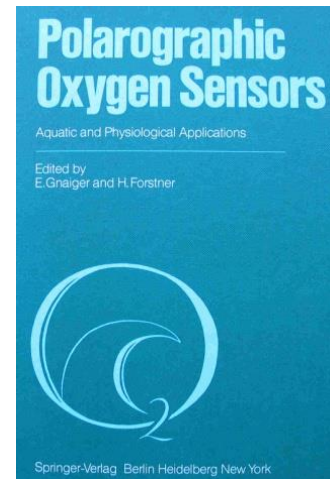
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**Summary:** High-resolution respirometry (HRR) critically depends on maintenance ([MiPNet19.18B](#)) and accurate calibration of the polarographic oxygen sensors (OroboPOS, POS). Calibration errors > 10 % as commonly encountered in the literature cannot be accepted in HRR. Standard operating procedures (**O2k-SOP**) are described: (1) Cleaning and preparation for use of O2k-chambers ([MiPNet19.03](#)); (2) Quality control for evaluation of proper POS function (SOP: O<sub>2</sub> sensor test, MiPNet06.03); and (3) Accurate POS calibration ([MitoPedia: O2-Calibration - DatLab](#), MiPNet06.03). This is Part 1 of O2k Quality Control, a component of the *Oroboros Diagnostics Quality Management*.

## 1. Calibration and quality control (O2k-SOP)

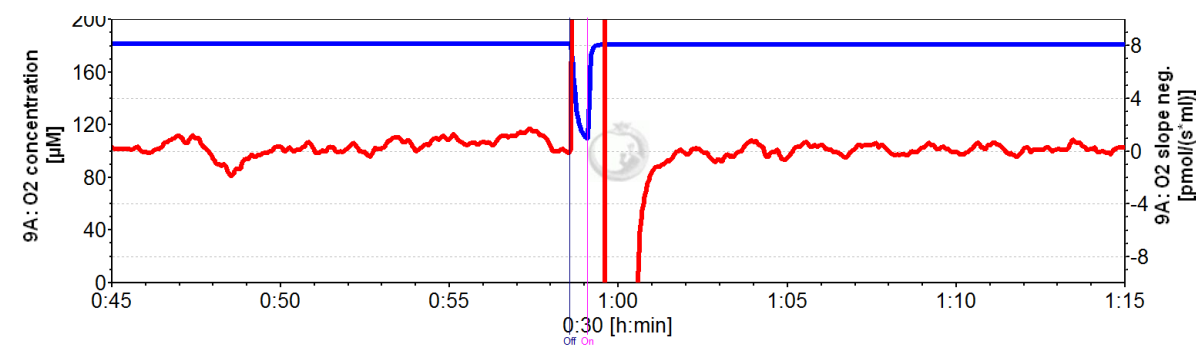
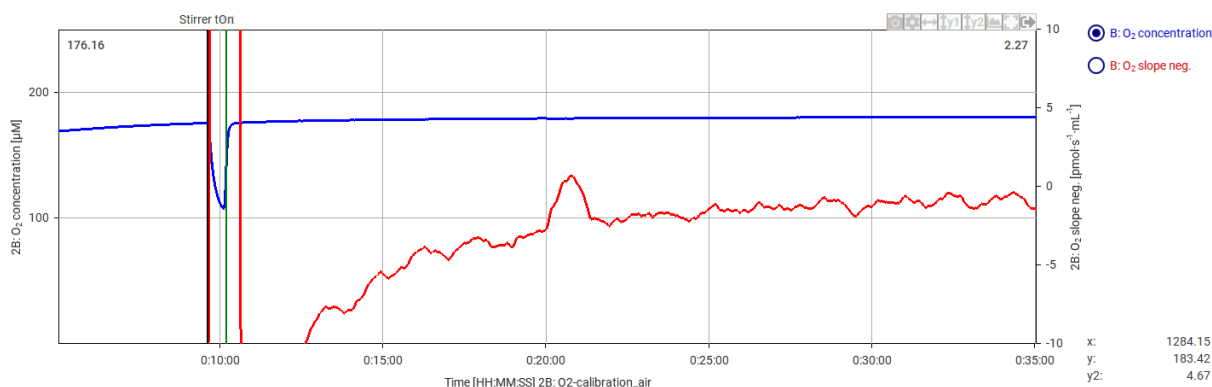
### 1.1. Preparation

1. Switch on the O2k, connect it to DatLab. Edit the O2k channel settings in DatLab. Clean the O2k-chambers using the DL-protocol "O2k-cleaning BeforeUse" ([MiPNet19.03](#)).
2. Increase the temperature of the stock of experimental medium to slightly above experimental temperature. Add 2.1-2.5 mL medium to each 2.0-mL O2k-chamber and 0.54 mL to each 0.5-mL O2k-chamber. This helps avoid the formation of gas bubbles and minimizes disturbance of the O2k temperature.
3. With the stirrer on (typically 750 rpm = 12.5 Hz), insert the stopper fully; check that no air bubbles are contained in the volume-calibrated chamber.
4. Siphon off excess medium from the top of the stopper.
5. Lift the stopper to the stopper spacer position.
6. Start a new file in DatLab and select the DL-protocol "O2-calibration air" or "O2-calibration air and zero".

### 1.2. The O<sub>2</sub> sensor test

**1. Stirrer test:** Even before final stabilization of O<sub>2</sub> slope neg., perform a stirrer test [F9], switching both stirrers automatically off and on. The default period is 30 s, for experiments at 37 °C. At lower experimental temperature, this period should be prolonged (60 s at 25 °C).

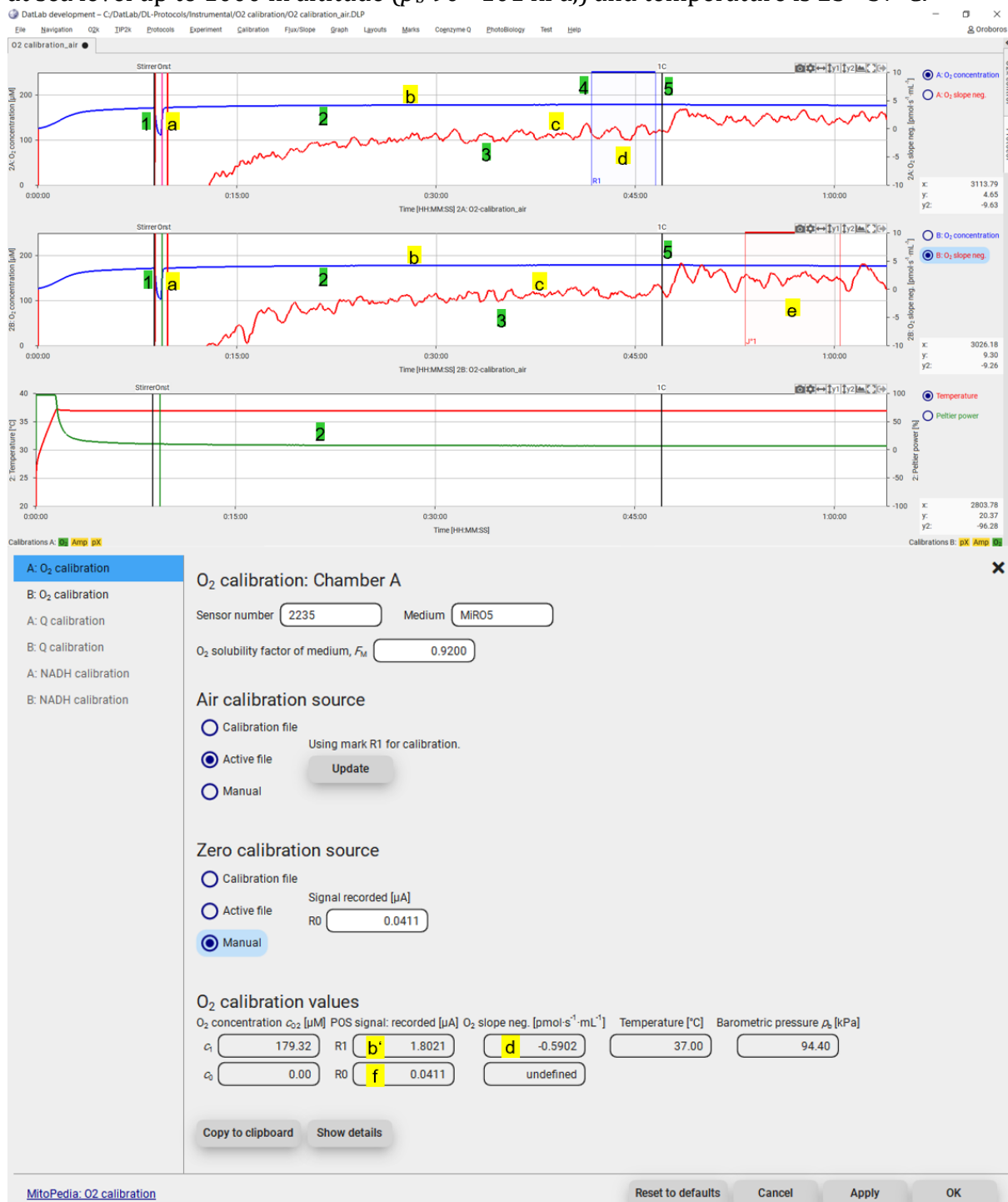
**Quality control a:** Upon automatic re-start of the stirrer (On), the increase of the oxygen signal should be rapid and monoexponential.



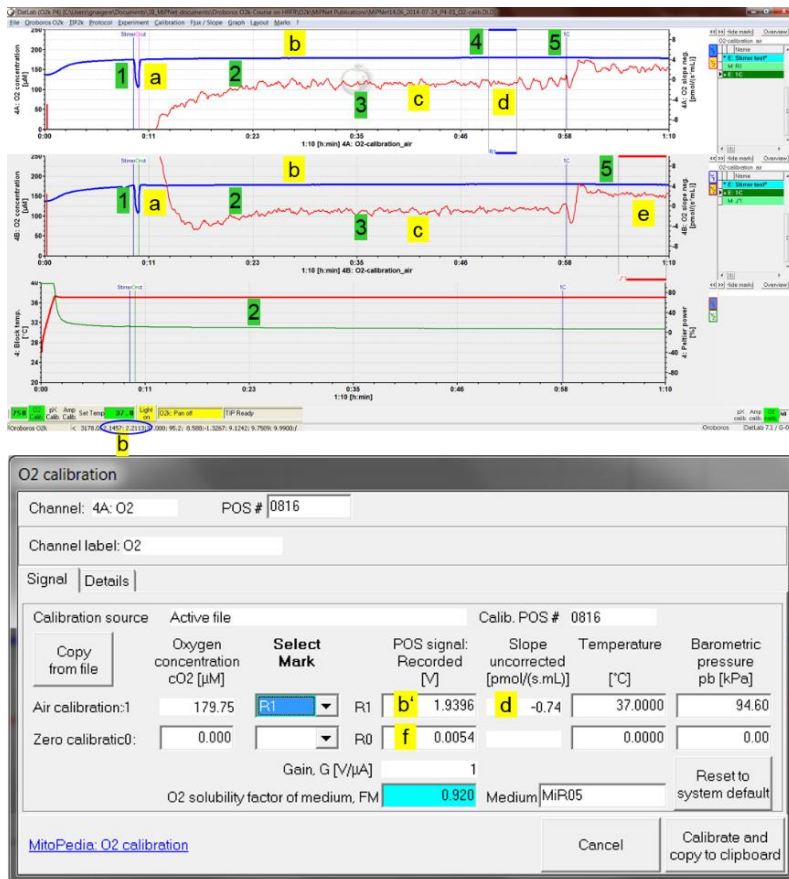
**1. Stirrer test for quality control of the POS** (standard 30 s) with 30 min time scale displayed with Graph Layout "O2-Calibration - Background" (MiR05; 37 °C; data recording interval: 2 s. **(A) DatLab 8** slope smoothing: 80 s. 2021-09-09 P2-01.DLD; **(B) DatLab 7.4;** slope smoothing: 40 data points. 2014-02-19 P9-01.DLD

2. About 20 min are required for approximate air equilibration after temperature equilibration of the incubation medium, visualized as stabilization of the Peltier power.

**Quality control b:** The raw signal (blue trace) should be between 1 and 3  $\mu\text{A}$  when at sea level up to 1000 m altitude ( $p_b$  90 - 101 kPa,) and temperature is 25 - 37°C.



**POS Quality control** (DatLab 8) using the DatLab protocol (DL-Protocol) O2\_calibration\_air.DLP: Plot of the O<sub>2</sub> sensor test (above; File 2017-02-06 P2-01.DLD; time scale is 1:05 h:min) and oxygen calibration window (below).



**POS Quality control (DatLab 7) using the DatLab protocol (DL-Protocol) O2\_calibration\_air.DLP:** Plot of the 1-hour POS performance test (above; File 2014-07-24\_P4-01\_O2-calib.DLD; time scale is 1:10 h:min) and oxygen calibration window (below).

from mark R1 of the current file; (3) clicking on 'Manual', allows for the values to be entered manually. If "Active file" is selected, the R1 will be shown in **b'**. Add the O<sub>2</sub> solubility factor of the medium,  $F_M$  (O<sub>2</sub> solubility factor).

**Quality control d:** The slope uncorrected should be  $\pm 1$  pmol·s<sup>-1</sup>·mL<sup>-1</sup> for 2.0-mL and 0.5-mL chambers if averaged across the section of the experiment marked as R1 for air calibration (**d**). The recorded POS signal should be close to the previous calibration under identical experimental conditions. Click on **Apply**.

**5** Close the chamber and if required, perform a zero oxygen calibration (section 4), or continue with a complete instrumental O<sub>2</sub> background test (MiPNet14.06).

**Quality control e:** After closing the chamber, select plot Y2 and set mark J<sup>o</sup>1. The O<sub>2</sub> slope (neg.) should be  $3 \pm 1$  pmol·s<sup>-1</sup>·mL<sup>-1</sup> for the 2.0-mL chamber (MiPNet14.06) and  $10.0 \pm 4$  pmol·s<sup>-1</sup>·mL<sup>-1</sup> for the 0.5-mL chamber. Flux values higher than 4.0 or 14.0 pmol·s<sup>-1</sup>·mL<sup>-1</sup> for the 2.0- or 0.5-mL chamber, respectively, may indicate a biological contamination.

**Quality control f:** The zero signal at mark R0 obtained at zero calibration (section 4) should be < 2 % of R1 (stable at < 5 % is acceptable).

**3.** Within 40 min, the oxygen signal should be stable with O<sub>2</sub> slope neg. (uncorrected) close to zero.

**Quality control c:** Signal noise should be low, reflected in the noise of the O<sub>2</sub> slope neg. (red trace) within  $\pm 2$  ( $\pm 4$  is acceptable) pmol·s<sup>-1</sup>·mL<sup>-1</sup> for both 2.0-mL and 0.5-mL chambers at a data recording interval of 2 s and slope smoothing 80 s selected for calculation of the slope.

**4.** Set a mark on the oxygen signal (R1 – the mark is automatically named R1 when using the DL-protocol) and click on **Calibration** to open the DatLab O<sub>2</sub> calibration window. Three options are available for applying O<sub>2</sub> calibration values:

(1) clicking on "calibration file" loads the values from a calibration file; (2) clicking on 'Active file', uses the values

## 2. Zero oxygen calibration

Zero calibration can be done by one of the following methods:

### 2.1. Zero calibration with instrumental O<sub>2</sub> background test: TIP2k

**O2k-SOP:** » [MiPNet14.06 Instrumental O2 Background](#)

### 2.2. Zero calibration: manual titration of dithionite (O2k-SOP)

1. Prepare "zero solution": Dissolve two spatula tips or 20 mg of dithionite (sodium hydrosulfite, Na-dithionite, Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub>; [O2-Zero Powder](#) in the [OroboPOS-Service Kit](#)) in 0.5 mL deionized water. Mix in a small vial with minimum gas space. Use fresh dithionite. Dithionite is oxidized during prolonged storage and needs to be replaced.
2. Inject 20 µL zero solution into the closed O2k-chamber using a [50 µL microsyringe](#).
3. Oxygen depletion is very rapid, and zero oxygen is reached within a few minutes. However, a few more minutes may be required until a stable signal is obtained,  $R_0$  [µA].
4. Inject another 10 µL zero solution. Repeat as long as the signal responds by a further decline. Siphon off excess medium from the stopper.
5. On the final titration when no further decline is seen, the zero signal stabilizes quickly ( $< \pm 0.2$  or  $< \pm 0.8$  pmol·s<sup>-1</sup>·mL<sup>-1</sup> for the 2.0-mL or 0.5-mL chamber respectively).
6. Set a mark over the stable "zero" signal ( $R_0$ ), to complete the two-point oxygen calibration [F5]. Select Mark **R1** and Mark **R0** for  $R_1$  and  $R_0$  in the O<sub>2</sub> calibration window.

### 2.3. Zero calibration: mitochondrial respiration

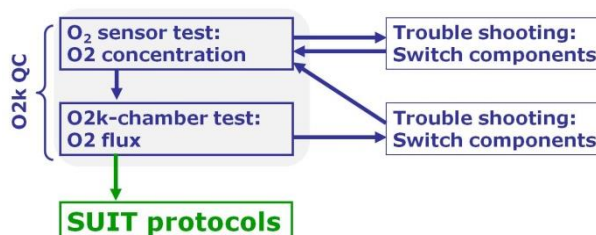
Due to the high oxygen affinity of isolated mitochondria, intact cells and tissue homogenate, residual traces of oxygen are insignificant after respiratory oxygen depletion. Therefore, you can use your experimental sample for such zero-oxygen calibration. Alternatively, prepare a stock of baker's yeast, with 200 mg dry yeast in 2 mL physiological salt solution. Stir heavily to obtain a homogenous suspension of yeast cells and add 50 µL yeast suspension into the 2-mL chamber through the cannula of the stopper, using a microsyringe.

**More details:** » [Gnaiger et al \(1995\)](#), » [Gnaiger \(2001\)](#).

## 3. O<sub>2</sub> sensor test and zero oxygen calibration: when?

The O<sub>2</sub> sensor test should be performed:

- Every day after switching on the O2k.
- During troubleshooting procedures, when switching components between the two chambers, a quick sensor test is performed after each step (stirrer test, raw sensor signal).





- After application of a new membrane and POS Service. In some cases, the signal of the OroboPOS improves (higher signal stability, less noise, shorter response time), when leaving the O2k switched on overnight (O2k-chambers filled with deionized H<sub>2</sub>O at 25° C with stopper in closed position and the illumination switched off).



Zero oxygen calibration should be performed:

- Every few weeks.
- When working at low oxygen levels it is recommended to measure R<sub>0</sub> before and after each measurement using the experimental sample for zero calibration (Section 2.3).

O<sub>2</sub> sensor test and zero calibration are also performed at the beginning and end of the O2k-chamber test (instrumental O<sub>2</sub> background test).

#### 4. O<sub>2</sub>-Calibration list: quality control



**Oroboros FileFinder:** Click on the icon “O2k-Manual”. Go to ‘O2k-Quality control and SOPs’ and move to the right to open the Excel file “O2-calibration.xlsx”. Save a copy of this Excel template and paste the calibration parameters into new lines sequentially for chamber (A) and (B), thus generating a data base for quality control of instrumental calibration (Gnaiger 2008). Trends over time can thus be evaluated, and possible irregularities of sensor performance are quickly recognized for intervention by sensor service.

» [MiPNet19.18B POS-service](#)

#### 5. O<sub>2</sub> calibration window - details

Concentration					
Calibration factor for concentration [ $\mu\text{M}\cdot\mu\text{A}^{-1}$ ]	$F_c$	<input type="text" value="99.7071"/>	$F_c = (c_1 - c_0) / (R_1 - R_0)$		
Calibration offset [ $\mu\text{A}$ ]	$a_c$	<input type="text" value="0.0037"/>	$a_c = (c_1 \cdot R_0 - c_0 \cdot R_1) / (c_1 - c_0)$		
Pressure					
	Oxygen pressure, $p_{\text{O}_2}$ [kPa]	POS signal: Current, $I$ [ $\mu\text{A}$ ]	Oxygen consumption by POS, $J_{\text{O}_2}$ [ $\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{mL}^{-1}$ ]		
Air calibration:	$p_1$ <input type="text" value="18.4587"/>	$I_1$ <input type="text" value="1.8022"/>	$J_1$ <input type="text" value="2.3300"/>	$I_1 = R_1$	$J_1 = 2.591 \cdot (I_1 - a_p) / V$
Zero calibration:	$p_0$ <input type="text" value="0.0000"/>	$I_0$ <input type="text" value="0.0037"/>		$I_0 = R_0$	
Calibration factor for pressure [ $\text{kPa}\cdot\mu\text{A}^{-1}$ ]	$F_p$	<input type="text" value="10.2634"/>	$F_p = (p_1 - p_0) / (I_1 - I_0)$		
Calibration offset [ $\mu\text{A}$ ]	$a_p$	<input type="text" value="0.0037"/>	$a_p = (p_1 \cdot I_0 - p_0 \cdot I_1) / (I_1 - I_0)$		
O <sub>2</sub> solubility, $S_{\text{O}_2}$ [ $\mu\text{M}\cdot\text{kPa}^{-1}$ ]	<input type="text" value="9.7148"/>	$c_{\text{O}_2} = p_{\text{O}_2} \cdot S_{\text{O}_2}$			
H <sub>2</sub> O vapor pressure, $p_{\text{H}_2\text{O}}$ [kPa]	<input type="text" value="6.2749"/>	$p_{\text{O}_2}^* = (p_b - p_{\text{H}_2\text{O}}) \cdot 0.20946$			
Volume fraction of O <sub>2</sub> in dry air	<input type="text" value="0.20946"/>				
O2k chamber volume, $V$ [mL]	<input type="text" value="2.0000"/>				
Gain	<input type="text" value="1"/>				

**Figure 3. O<sub>2</sub> calibration window – details (DatLab 8).** Upon clicking ‘show details’ in Calibration window oxygen calibration parameters are displayed as calculated by DatLab. (For DatLab 7, see Supplement).

In the calibration window click on “view details” to see the oxygen calibration parameters and equations calculated by DatLab.

**Concentration:** Parameters are displayed for conversion of the raw signal to concentration.

**Calibration factor for concentration,  $F_c$  [ $\mu\text{M}\cdot\mu\text{A}^{-1}$ ]:** This is the multiplication factor,  $F_c$ , calculated to convert the recorded current (corrected for the zero signal) into oxygen concentration (Supplement C - Eq. 2).

**Calibration offset,  $a_c$  [ $\mu\text{A}$ ]:** This is the POS zero signal at zero oxygen concentration, which is subtracted from the current before multiplication with the calibration factor (Supplement C - Eq. 3).

**Pressure:** Parameters are displayed for conversion of the POS signal current to partial pressure of oxygen. These are the fundamental parameters for evaluation of signal stability over periods of several months since the POS responds to partial pressure of oxygen in the medium rather than concentration.

**$p_1$  [kPa]:**  $p_{\text{O}_2}$  at air saturation,  $p_{\text{O}_2}^*$ , a function of temperature and barometric pressure.

**$p_0$  [kPa]:** Usually  $p_{\text{O}_2}$  at zero oxygen concentration, or any other  $p_{\text{O}_2}$  at the second calibration point,  $p_0$ .

**$I_1$  [ $\mu\text{A}$ ]:** POS signal as a current, at air saturation.

**$I_0$  [ $\mu\text{A}$ ]:** POS signal as a current, at zero oxygen concentration, or any other  $p_{\text{O}_2}$  at the second calibration point.

**Oxygen consumption by the POS,  $J^{\circ}\text{O}_2$  [ $\text{pmol}\cdot\text{s}^{-1}\cdot\text{mL}^{-1}$ ]:** Theoretical oxygen consumption of the oxygen sensor at air saturation under experimental conditions (Supplement C - Eq. 9).

**Calibration factor for pressure,  $F_p$  [ $\text{kPa}\cdot\mu\text{A}^{-1}$ ]:** This is the multiplication factor,  $F_p$ , calculated to convert the current of the POS (corrected for the zero current) into partial pressure of oxygen (Supplement C - Eq. 5).

**Calibration offset,  $a_p$  [ $\mu\text{A}$ ]:** This is the POS zero current, at zero partial pressure of oxygen, which is subtracted from the current before multiplication with the calibration factor (Supplement C - Eq. 4).

**$\text{O}_2$  solubility,  $S_{\text{O}_2}$  [ $\mu\text{M}\cdot\text{kPa}^{-1}$ ]:** a function of temperature and oxygen solubility factor of the medium (Supplement C - Eq. 7).

**$\text{H}_2\text{O}$  vapor pressure,  $p_{\text{H}_2\text{O}}^*$  [kPa]:** a function of temperature, is subtracted from the barometric pressure,  $p_b$ . (Supplement C - Eq. 9).

**Volume fraction of  $\text{O}_2$  in dry air:** 0.20946, when multiplied with the pressure ( $p_b - p_{\text{H}_2\text{O}}^*$ ), it yields the partial pressure of oxygen.

**$\text{O}_2\text{k}$  chamber volume,  $V$  [mL]:** The effective aqueous volume of the closed  $\text{O}_2\text{k}$ -chamber.

**Gain,  $G$ :** The gain is used for current to voltage conversion. Gain is fixed to 1.

## 6. References

Gnaiger E (2001) Bioenergetics at low oxygen: dependence of respiration and phosphorylation on oxygen and adenosine diphosphate supply. *Respir Physiol* 128:277-97. » [Bioblast link](#)

Gnaiger E (2008) Polarographic oxygen sensors, the oxygraph and High-Resolution FluoRespirometry to assess mitochondrial function. In: *Mitochondrial Dysfunction in Drug-Induced Toxicity* (Dykens JA, Will Y, eds) John Wiley:327-52. » [Bioblast link](#)

Gnaiger E, Forstner H, eds (1983) *Polarographic Oxygen Sensors. Aquatic and Physiological Applications*. Springer, Berlin, Heidelberg, New York:370 pp. » [Bioblast link](#)

Gnaiger E, Steinlechner-Maran R, Méndez G, Eberl T, Margreiter R (1995) Control of mitochondrial and cellular respiration by oxygen. J Bioenerg Biomembr 27:583-96.  
»[Bioblast link](#)

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## Supplement: O2 calibration window – details (DatLab 7)

### O2 calibration

Channel: 9A: O2	POS # 0521
Channel label: O2	
Signal Details	
Concentration	
Calibration factor for concentration [ $\mu\text{M}/\text{V}$ ]	Fc 85.27 $F_c = (c1-c0) / (R1-R0)$
Calibration offset [V]	ac 0.0124 $a_c = (c1-R0-c0-R1) / (c1-c0)$
Pressure	
Oxygen pressure pO2 [kPa]	POS signal: Current I [ $\mu\text{A}$ ]
Air calibration: p1 18.647	I1 2.0678 $I1=R1/G$
Zero calibration: p0 0.0000	I0 0.0124 $I0=R0/G$
Calibration factor for pressure [kPa/ $\mu\text{A}$ ]	Fp 9.072 $F_p = (p1-p0) / (I1-I0)$
Calibration offset [ $\mu\text{A}$ ]	ap 0.0124 $a_p = (p1-I0-p0-I1) / (p1-p0)$
O2 solubility, S02 [ $\mu\text{M}/\text{kPa}$ ]	9.40 $cO_2 = pO_2 \cdot S02$
H2O vapor pressure pH2O* [kPa]	6.28 $pO_2^* = (p_b - pH_{2O}^*) \cdot 0.20946$
Volume fraction of O2 in dry air	0.20946
O2k Chamber volume, V [ml]	2.00
<a href="#">MitoPedia: O2k-Calibration</a>	
<div style="text-align: right;"> <input type="button" value="Cancel"/> <input type="button" value="Calibrate and copy to clipboard"/> </div>	

**Figure S1. O2 calibration window – details (DatLab 7).** Upon clicking [F5] / Tab Details (MiPNet26.06) oxygen calibration parameters are displayed as calculated by DatLab.